USE OF AUTOMATED TECHNOLOGY IN CHEMICAL PROCESS RESEARCH AND DEVELOPMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Patent Application No. 09/443,987 filed on November 19, 1999, which is a continuation of U.S. Patent Application No. 08/862,840 filed on May 23, 1997 (U.S. Patent No. 6,044,212), which is a continuation-in-part of U.S. Provisional Patent Application No. 60/018,282 filed on May 24, 1996. This application incorporates by reference U.S. Provisional Patent Application No. 60/018,282 in its entirety. This application also incorporates by reference U.S. Patent No. 6,044,212 in its entirety.

FIELD OF THE INVENTION

This invention relates to the use of automated technology in chemical process research and development, particularly for identifying and optimizing conditions for crystallizing molecules. Crystallization may take a variety of forms including without limitation: (1) crystallization of simple organic compounds from reaction mixtures; and (2) single enantiomers from racemic mixtures. This technology includes automated process methodology, product structural characterization and purity analysis, and computer-controlled design of experiments (DOE) planning and data interpretation. The invention represents a means by which chemical process identification and optimization, including crystallization of simple organic and complex molecules, can be greatly accelerated and more effectively conducted.

BACKGROUND OF THE INVENTION

Optimization of chemical process development is an important procedure whereby conditions are discovered to produce a chemical product efficiently, cost-effectively, safely, and with high quality assurance. One aspect of chemical process development is crystallization. Crystallization may come in a variety of forms depending on the needs of the chemical process (*e.g.*, purifying of a compound, etc.). Some

example of crystallization include: (1) crystallization of simple organic compounds from reaction mixtures; and (2) single enantiomers from racemic mixtures.

Regardless of the type of crystallization, optimization of the crystallization process is extremely complex. A variety of variables, many of which are time dependent, affect the crystallization process. Since these variables are interdependent, the possible combinations and permutations of these variables are numerous. As a result, an enormous effort must be undertaken to study the various combinations of variables in order to identify the optimal set of conditions for conducting a given crystallization process.

One such variable is temperature. Typically, lowering the temperature encourages crystallization; however, the temperature may need to be dynamically controlled such that it is modified during a crystallization experiment. Another variable is agitation of the sample. There are times when the mixing of the solution is required to dissolve the solute in the solvent. Again, agitation may be time dependent such that the agitation of the sample is performed only during the time in which it take to dissolve the solute and not during the time in which the crystals are forming (otherwise, the agitation may break the crystal structure apart). Another variable is pH. Still a further variable is gravity. Gravity may affect the crystalline structure, thereby affecting the ability of the desired compound to crystallize. Another variable relates to solvents. For example, crystallization is affected by the types of solvents selected, the solvent mixtures (mixtures of different types of solvents), and concentrations of solvents (such as the concentration of salt in an aqueous solution). There are typically a large number of solvents available for crystallization, As discussed in more detail below for the specific crystallization processes, the many combinations of these types of variable makes the process of optimization extremely difficult requiring a system and method for optimization.

A. Crystallization of Simple Organic Compounds From Reaction Mixtures

Crystallization of compounds, such as simple organic molecules and complex molecules (e.g., proteins and nucleic acids), is an important process in the chemical industry, particularly the pharmaceutical industry. This type of crystallization separates and purifies and/or resolves compounds from reaction and racemic mixtures, and for rational drug design. For example, if a particular compound is in a mixture with other non-desirable compounds, that particular compound may be "removed" from the mixture

by crystallization of the particular compound, and then by withdrawing the crystal from the mixture.

At present, small compounds and complex molecules such as proteins and nucleic acids are crystallized by a variety of conventional experimental methods. For simple organic molecules, some of the variables which affect crystallization include temperature, agitation and solvents (including types of solvents, solvent mixtures and concentrations of solvents). For example, there are a large variety of solvents/solvent mixtures/ concentrations of solvents which are available for crystallization. Identification of a suitable solvent system to selectively crystallize a target molecule is often tedious and time consuming, particularly if complex mixtures are involved.

B. Single Enantiomers From Racemic Mixtures

There is a growing need to develop efficient methods for producing single-enantiomer forms of molecules. Certain molecules, because of their chirality, bind to binding or receptor sites in proteins (*i.e.*, either the left or right hand molecule selectively binds to the particular binding site or receptor). This binding interaction can trigger or inhibit a cascade of biological reactions.

Single enantiomer molecules are typically acquired via one of three methods: separation of racemic mixtures using kinetic crystallization techniques, chiral chromatography, or asymmetric synthesis. While asymmetric synthesis is preferred for obtaining single enantiomers, efficient asymmetric syntheses of particular target molecules are not always achievable. In many instances, it is more economical to synthesize a racemic mixture and separate out the desired enantiomer by kinetic crystallization resolution using chiral crystallization partners or by chiral chromatography. The typical molecules that are resolved using kinetic resolution are molecules having either acidic or basic functional groups, that can be converted to salts with chiral bases or chiral acids, respectively. However, identifying a suitable chiral crystallization partner from myriads of potential partners is often tedious and time consuming.

In summary, the conventional practice of screening for optimal crystallization conditions involves a manual survey of different variables such as concentration, solvent selection, temperature, pH, time, etc., which is time consuming, labor intensive and

repetitive. The end result is that only a small percentage of the possible combinations of variables are investigated using the manual approach. It is thus highly desirable to develop highly efficient, simple, and effective methodologies for obtaining the desired conditions for the crystallization of molecules and yet which can also avoid the problems associated with the prior art methods.

SUMMARY OF THE INVENTION

The use of automated technology in chemical process research and development is disclosed. This technology is applicable to automated synthesis methodology, product structural characterization and purity analysis, and computer-controlled design of experiments (DOE) planning and data interpretation. The invention represents a means by which crystallization identification and optimization can be greatly accelerated and more effectively conducted.

BRIEF DESCRIPTION OF THE DRAWINGS

The following discussion will make reference to the accompanying drawing figures, wherein like reference numerals refer to like elements in the various views, and wherein:

FIG. 1 is a diagram of the components of a preferred workstation for implementing the invention;

FIG. 2 is a block diagram illustrating the flow of commands and data between the computer and synthesizer, robotic arm and product analyzer of FIG. 1;

FIG. 3 is flow chart illustrating the sequence of steps in performing the preferred crystallization optimization routine using the equipment of FIG. 1;

FIG. 4 is an additional block diagram of the computer, synthesizer, robot, and analyzer;

FIGS. 5A-5G are an additional flow chart of the sequence of steps in performing the preferred crystallization optimization routine using the equipment of FIG. 1; and

FIG. 6 is a block diagram illustrating the computational analysis, particularly diversity analysis, used to evaluate a large library of potential crystallization partners.

DETAILED DESCRIPTION OF THE INVENTION

A. System Overview

In this description, the novel application of automated technology to chemical process development is disclosed. The basic concept is to have a machine perform the repetitive procedures involved in process development in order to increase the efficiency with which data can be collected and analyzed for a given crystallization.

A preferred workstation for implementing the invention is shown in FIG. 1. The workstation 10 includes a synthesizer 12 having a block 14 having, for example, 48 wells 16. As discussed previously, several variables, including temperature, agitation, pH, solvents, etc., contribute to crystallization of a compound. The process of optimization iteratively determines the values for the variables to optimize crystallization.

The synthesizer 12 allows for ranges of temperature (including dynamic changes of the temperature during a given crystallization experiment), agitation (including dynamic changes in the amount of agitation and the timing of the agitation during a given crystallization experiment), etc. In particular, the synthesizer 12 preferably is equipped with a temperature control system 18 for adjusting the temperature of the block 14, so as to control the temperature of the wells 16. Preferably, the temperature control system 18 has the capability of controlling the temperatures of the wells individually, so that the conditions in the wells 16 can be customized. The synthesizer includes a lid or cover 20. A source 22 of gas (such as nitrogen or argon gas) is connected to the synthesizer 12 via a conduit 24, which enables a control of the atmospheric conditions above the wells. Mixing mechanisms such as a vortex mixer or an orbital shaker can be built into the synthesizer 12 to assist in the mixing or agitation of the solutes in the solvents in the wells.

As part of the dynamic control of fluids in the wells during a given crystallization experiment, the wells may include one or more fluid conduits that can allow fluids, such as solvents or buffers, to flow into and/or out of the well as desired. These conduits can be attached to one or more pumps to control the rate of solvent flow. This allows the operator to selectively change solvents or solvent mixtures or buffer concentration in a stepwise manner during crystallization.

The synthesizer 12 further includes a robotic arm assembly 26 which has pipetting capability for selectively adding quantities of one or more fluids (such as reagents and solvents) to the wells 16. The robotic arm assembly 26 includes an X-Y drive mechanism 28 or other suitable means for controlling the position of the pipetting tip portion 30 of the arm assembly relative to the wells. The pipetting tip portion 30 further includes equipment for monitoring the reactions in the wells 16 and for checking for crystallization. The monitoring of the wells may be performed at periodic intervals for a predetermined amount of time. A synthesizer capable of operating experiments is by Bohdam Automation (Mundelein, Ill.) which features the automated synthesis workstation capable of solid-phase and solution-phase synthesis, performing upwards of 48 simultaneous crystallizations. The reactions can run in an atmosphere and solvent of choice by the operator at temperatures ranging from –40°C. to +150°C.

The station 10 also includes an analytical instrument 40, such as a microscope, near IR spectrophotomer, a polarimeter, an HPLC or LC/MS for conducting analysis of the products of such crystallization. The analysis of the experiments determines whether a particular experiment is a "success" or a "failure." The criteria for "success" or failure is varied and includes, without limitation, whether the experiment produced a crystal, what is the purity of the crystal, what is the yield of the crystallization, and/or what is the quality of the crystal. Depending on the criteria, various instruments may be used. For example, in order to determine whether a specific experiment has crystallized, a microscope may be used to examine the sample. In an alternate embodiment, manual inspection (*e.g.*, visual inspection) of the sample may be performed in place of, or in combination with, the analytical instrument to determine whether the experiment crystallized. As another example, to determine the purity of the crystal structure, the crystal produced from the experiment may be analyzed using NMR (nuclear magnetic resonance), near IR spectrophotometer, HPLC, etc. As discussed in more detail below, the purity of the crystal generated should be analyzed. If there is co-crystallization (crystallization of the desired compound and another undesired compound), this may effect whether the experiment was a "success" or "failure." In addition, if enantiomeric purity is sought, a polarimeter may be used. If the criteria for "success" or "failure" depends on yield, the purity of the crystal may first be analyzed (to determine the amount

of the crystal which is composed of the desired compound). Thereafter, the crystal may be weighed using a scale and the percentage yield may be calculated based on the amount of the desired compound which was introduced into the specific experiment. If the criteria for "success" or "failure" is the form of the crystal (*e.g.*, single unbroken crystal being desired), a mass spectrometer may be used as an analyzer to analyzed the form of the crystal.

The crystallization products from the synthesizer 12 can be either manually loaded into the analytical instrument 40, or loaded automatically with the assistance of suitable robotic arms or other equipment, represented by robot 50 in FIG. 2 or other suitable mechanical system.

The operation of the synthesizer 12 and analytical instrument 40 is controlled by a computer 42, as shown in the block diagram of FIG. 2. The computer 42 regulates the environmental conditions in the synthesizer 12, such as by controlling the temperature of the wells 16 and controlling the agitation within the wells 16. The quantity and type of reagents, such as buffer solutions, crystallization partners, and the quantity and type of solvents added to the wells is also controlled by the computer 42, as is the position of the arm 26 relative to the wells 16. The computer 42 may dynamically control the environmental conditions or the fluids introduced into or removed from the wells 16. This dynamic control may be set prior to initiation of the experiment. For example, the agitation of the wells may be programmed prior to the experiment for a predetermined period (*e.g.*, the first 2 minutes of the experiment). Alternatively, the dynamic control may be based on analysis of the progression of the experiment. For example, the analyzer 40 may periodically monitor the well to determine if crystallization has begun. If the analyzer 40 senses that crystallization has begun, the environmental conditions or other variables may be modified (such as modifying the temperature in the wells, ceasing agitation within the wells, and/or changing the solvents in the wells).

The computer 42 further initiates and controls the analysis of the crystallization products in the analytical instrument 40, and receives the analytical data from the instrument 40. The computer 42 further implements a design of experiment (DOE) program that is used to identify the optimal conditions for the crystallization being studied, as described below. It will be understood that some or all of the control functions

of the computer 42 may be integrated into one or more of the individual components of the system 10. Where the crystallization products are automatically loaded into the product analyzer 40, the computer 42 controls a robot 50 to perform this task.

An additional block diagram of the computer, synthesizer, robot, and analyzer is shown in FIG. 4. The computer 42 contains a processor 64 which communicates with non-volatile (read only memory, ROM 68) and volatile (random access memory, RAM 70) memory devices. The processor 64 also has a comparator 66 for comparing values. The processor 64 executes a computer program, as described subsequently in FIG. 5. The computer program is stored in the ROM 70 and executed either in the RAM 68 or the ROM 70.

The processor 64 communicates with various subcomponents of the synthesizer 12, the analyzer 40 and the robot 50. The synthesizer contains a temperature control system 18 which controls the temperature of each of the individual wells of the block. The processor sends a command to the temperature control system 18 specifying a certain temperature for a particular well. The synthesizer also contains an agitator/mixer 76 which agitates or mixes the individual wells. There are two different methods of agitating or mixing. The first method is to agitate the block as a whole whereby each of the wells are shaken at the same rate. To do this, the entire block is agitated at one rate. The second method is to mix each of the individual wells at different rates. Each well is equipped with at metal stirrer underneath the well. Inside the well is a TEFLON®-coated magnet which follows the motion of the metal stirrer underneath the well. In this manner, the individual well is stirred based on the rate at which the metal stirrer is rotated. The rate of rotation is set by the processor 64.

The synthesizer also contains an atmospheric regulator 78 which protects the components in the wells if the components are sensitive to oxygen or water or other materials in the environment in proximity to the well. Nitrogen or argon gas is dispensed from the source 22 through the conduit 24 based on a valve which is controlled by the valve motor 80. The valve motor is controlled by the processor 64.

The synthesizer further contains a drive 28 for moving the robotic arm assembly 26. As described above, the robotic arm assembly 26 has pipetting capability for selecting, obtaining and dispensing one or more reagents or solvents. The pipetting

capability is performed through a pipetting mechanism 74 which draws reagents and/or solvents through the pipetting tip portion 30 and stores one or more reagents in the robotic arm assembly 26. Subsequently, the one or more reagents and/or solvents are dispensed via the pipetting mechanism 74 into the wells. Both the drive 28 and the pipetting mechanism 74 are controlled by the processor 64.

The analyzer 40 and robot 50 are in communication with the processor 64 as well. The processor 64 controls the drive 72 of the robot 50 which extracts samples from each of the wells. The samples are transferred to the analyzer 40 which analyzes the contents of the sample such as the components of the crystallization mixture including the crystal product, the starting material, and any contaminants.

B. Methodology

Where the number of crystallization partners and solvents are in the hundreds, hundreds of thousands of different solvent combinations and/or crystallization partners are possible. The practical consequence is that expanding the numbers of compounds under evaluation increases the probability of discovering a particular solvent mixture that produces high quality crystals. Testing of combinations of solvents is done, with further testing performed based on interpretation of the results of the prior tests. In this manner, optimization of the conditions used in crystallizing the compound through an iterative process of running tests, interpreting the tests and generating new parameters of testing for future tests based on the analysis of the current tests.

Automated process development is distinctly advantageous over manual surveys of process conditions. The automated process is capable of executing significantly more tests at one time with less operator input. Further, the automated process development assists the operator by analyzing the test results and suggesting parameters for further testing. As discussed previously, different experiments have different criteria for determining whether a particular experiment is a "success" or a "failure." These optimal conditions are defined by the operator for the particular test. Ordinarily, conditions of interest to an operator include: amount of yield; crystallization yield, crystal quality, amount of uncrystallized material, enantiomeric purity of the crystals, temperature and time of crystallization.

A preferred automatic chemical process development technique according to the present invention is shown in flow-chart form in FIG. 3, and will be described in conjunction with the system shown in FIG. 1. The synthesizer 12 containing a 48-well block 14 is used for the crystallization of interest, and the robotic arm 26 can be programmed to dispense precise amounts of reagents and solvents into each well (see FIG. 1). Each well 16 contains a separate experiment. The temperature within each well can be controlled and the contents of each well can be efficiently mixed. As an example of one possible study, with a 48-well block 14, twelve different solvent systems at four different concentrations can be investigated. As shown at block 52, the solvents and reagents are dispensed in the wells. As shown at block 54, the 48 crystallizations are then run simultaneously in the time that only four crystallizations could be run in a manual approach. As shown at block 56, the crystallization mixture can then be filtered and crystals isolated and/or aliquots may be removed using the same robotic technology. This process alleviates the chemist from performing repetitive tasks and increases the efficiency with which information can be gathered.

Once the 48 crystallizations are completed, at step 4, as shown at block 58 of FIG. 3, the tasks of crystallization analysis and data compilation begin. These processes can also be automated. The success of each of these 48 crystallizations can be evaluated using the analytical technique which was already developed for the parent crystallization. For example, HPLC might be the analytical method of choice. In this case, the crude product mixtures would be manually or automatically transferred to wells which fit in an HPLC autosampler 40. Alternatively, other instruments may be used for analysis including microscope, near IR spectrophotomer, a polarimeter, or LC/MS. Analysis of each crystallization mixture would be completed automatically and the results would be compiled and analyzed by the computer 42. This computer 42 also controls the synthesizer 12 and the HPLC unit 40.

At this point (step 5, block 60), the chemist would determine how to interpret the experimental data. If product yield is the primary concern, this can be calculated by quantitative analysis from HPLC data. Alternatively, the chemist may be interested in the reaction conditions which minimize the co-crystallization of an undesirable contaminant such as a undesired enantiomer, maximize a particular crystal form, or result in X-ray

diffraction quality crystals. In such cases, near IR would be useful to gain additional information about the new crystal forms. Polarimetry would be an analytical method used where crystallizations to separate enantiomeric compound mixtures are involved.

The concept of statistical design of experiments (DOE) may be applied to aid in experimental design (step 6, block 62). Commercially available computer programs can, in fact, control the crystallization conditions utilized by the synthesizer to conduct the most effective DOE study. The computer 42 can then correlate the data obtained on crystallization yield, product purity, etc. and extrapolate to propose, and subsequently confirm, optimal crystallization conditions. This is represented by the arrow 51 in FIG. 3. Basically, a new and more narrowly circumscribed set of crystallization conditions are programmed in the synthesizer and robotic arm, and the process is repeated. This procedure could iterate several times, until the optimal crystallization conditions are determined with the desired level of precision. Alternatively, the procedure (steps 1-5) could just be performed once, with the computer 42 identifying which of the wells 16 had the most favorable conditions for the crystallization.

FIGS. 5A-5G are an additional flow chart of the sequence of steps in performing the preferred crystallization optimization routine. The program which executes the operation of the automated sequence of operations, as stated above, is resident either in RAM 68 or ROM 70. The program first determines the initial values of components for the experiments. The components include both solvents (the medium which allow for crystallization) and reagents (the compound to be crystallized (or the compound to be purified) and the crystallization partners (which aid in crystallization)). In particular, the program determines the initial values of the solvent concentrations, types of solvents, and solvent ratios for each of the wells, and the initial values of the reagent concentrations, types of reagents, and reagent ratios for each of the wells as shown at block 82. This is done so that the processor 64 can command the pipetting mechanism 74 to obtain the correct solvents and reagents and the approximate amount of solvents and reagents for use in all of the wells. As shown in FIGS. 5A-5G, the total number of wells is designated as "X." As discussed above, one block 14 has, for example, 48 wells 16. Blocks with less or more wells may be used as well.

The processor 64 then instructs the drive 28 to a particular x and y position to obtain the components (both solvents and reagents), as shown at block 84. The pipetting mechanism 74 then stores the components (solvents and reagents) in the dispenser of the drive of the synthesizer 12, as shown at block 86 of FIG. 5. Then a loop is executed for each of the wells 16, with the well_number set equal to 1, as shown at block 88 of FIG. 5. The processor 64 moves the motor of the drive 28 to the x and y position of the well 90, the component values and type of component is determined by the processor 92, and the components (solvents and reagents) are dispensed into the well, as shown at block 94 of FIG. 5. The component values and type of components are determined by a parameter look-up table 69 (which contains all of the relevant parameters for the experiment) in the memory of the microprocessor. The component values and type of components are either based on operator input or based on the optimization scheme described subsequently. The well_number is incremented by 1, as shown at block 96 of FIG. 5. If the well_number is greater than the total number of wells (X), then the loop is exited, as shown at block 98 of FIG. 5. Otherwise, the flow chart of FIG. 5 goes to block 90.

Alternatively, the pipetting mechanism, rather than storing the components (solvents and reagents) in the dispenser in one step and dispensing in another step may alternatively store the solvents and dispense, sequentially for each well and then store the reagents and dispense, sequentially for each well. Further, rather than automatic obtaining and dispensing of the components (solvents and reagents), the operator may manually input the components (solvents and reagents) values into the wells.

Prior to execution of the program, a component-properties look-up table is created which determines, for a specific component, whether the component is sensitive to oxygen or water. This component-properties look-up table may be separate and distinct from the parameter look-up table 69, or may be combined for operator convenience. Based on the component-properties look-up table, if the component is sensitive to oxygen or water 100, the processor 64 opens the valve motor 80 to dispense either nitrogen or argon gas, as shown at block 102 of FIG. 5. The well_number is set equal to 1, as shown at block 104 of FIG. 5. Then, the clock for the processor 64 is checked with the value stored as the start_time of the experiment 106. A loop is then entered to set the temperatures of each of the wells. The temperature is determined for each well 108 by the

parameter look-up table 69. The temperature in the parameter look-up table 69 is either based on operator input or based on the optimization scheme described subsequently. The processor 64 sends a command to the temperature control system 18 to set the temperature value 110. The well_number is incremented by 1, as shown at block 112 of FIG. 5. If the well_number is greater than the total number of wells (X), then the loop is exited, as shown at block 114 of FIG. 5. Otherwise, the flow chart of FIG. 5 goes to block 108. Moreover, as described previously, the temperature may be dynamically controlled in that the temperature may be modified based on a predetermined event (*e.g.*, modify the temperature at the first indication of crystallization).

The agitation/mixing of the synthesizer is next initialized based on whether the individual wells are mixed at different rates or whether the entire block is agitated at the same rate, as shown at block 116 of FIG. 5. If the agitation is at the same rate, the program determines the block agitation from the parameter look-up table 118 and sends a command to the agitator/mixer 120. If the agitation is at different rates, the program enters a loop, with the well_number set equal to 1 as shown at block 122 of FIG. 5, and determines the agitation from the parameter look-up table for each well 124 and sends a command to the agitator/mixer 126. The well_number is incremented by 1, as shown at block 128 of FIG. 5. If the well_number is greater than the total number of wells (X), then the loop is exited, as shown at block 130 of FIG. 5. Otherwise, the flow chart of FIG. 5 goes to block 124. Moreover, as described previously, the agitation may be dynamically controlled in that the agitation may be modified based on a predetermined event (*e.g.*, cease agitation at the first indication of crystallization).

The crystallization times are then determined for each of the wells, as shown at block 132 of FIG. 5, based on data in the parameter look-up table 69. The wells are ordered in an array based on the crystallization time, from lowest to highest with a pointer set to the first item in the array, as shown at block 134 of FIG. 5. The crystallization time is determined for the well which is at the pointer, as shown at block 136. The crystallizations are then checked periodically based on checking the clock from the processor 64 and subtracting the time from the start value 138. Aliquots of the crystallization mixture can be removed periodically to determine if crystallization has occurred. If so, modification can be made to the operating conditions (*e.g.*, increasing or

decreasing the temperature, ceasing or beginning agitation, etc.), solvents (*e.g.*, modifying the types of solvents or solvent concentrations, etc.), and/or reagents (increasing or decreasing the concentration of the desired compound for crystallization, increasing or decreasing the crystallization partners, or adding new crystallization partners). When the crystallization time has been exceeded for a particular well, as shown at block 140, the crystallization is stopped 142. Alternatively, crystallization may be stopped if it has been determined that crystallization has already occurred. In still an alternate embodiment, crystallization may be stopped if the amount of crystallization is stable over a predetermined period of time. For example, in chiral resolution, if the amount of chiral resolution is stable over a predetermined period of time (*i.e.*, removing aliquots from the wells does not produce greater chiral resolution), crystallization may be stopped.

Stopping the crystallization can be done in several ways including filtering the mixture or siphoning out the solvent or buffer. The pointer is set to the next item in the array, as shown at block 144. As shown at block 146, if the pointer is outside of the array, the flow chart goes to block 152. Otherwise, the flow chart goes to block 136. Then, based on the parameter look-up table 69, the processor determines whether to interrupt the entire block 148. If yes, the block is interrupted, as shown at block 150.

The well_number is set equal to 1, as shown at block 152 of FIG. 5. The processor 64 signals the drive 72 of the robot 50 to move to an x and y position 154, extract mixture from the well 156, and send the mixture to the analyzer 158. The analyzer 40 then analyzes the components of the crystallization mixture, as shown at block 160, and sends the results to the processor 64. In one embodiment of the invention, after the crystallization, at least one component from each of the wells 16 is removed, sent to the analyzer 40 and analyzed. For example, if optimization of chiral resolution is desired, aliquots of the solution (which has not crystallized) in the wells is analyzed to determine the magnitude of chiral resolution. One method for determining the magnitude of chiral resolution is measuring the amount of optical rotation. This may be performed by a polarimeter. Alternatively, a chiral HPLC machine may be used to determine the amount of chiral resolution.

In another embodiment of the invention, the wells are analyzed (with or without removing components from the wells). For example, to determine whether crystallization has occurred, the wells may be visually inspected. Alternatively, the solution in the wells may be filtered and examined under a microscope. If the quantity of the crystals is at issue, the yield of the crystals may be determined. For example, a percentage may be calculated of the amount of the target compound that crystallized divided by the amount of the target molecule introduced into the well. If the quality of the crystals is at issue, the crystals may be examined visually to determine the size and/or color of the crystals. Alternatively, the crystals may be examined using HPLC or mass spectroscopy to determine whether compounds other than the target molecule are included in the crystal (*i.e.*, impurities).

The processor 64 examines the data from the analyzer 40 and, in one embodiment, determines the yield in each of the wells as discussed previously, as shown at block 162. Some analyzers perform this look-up table function itself and send the list of products back to the processor. The processor stores the analysis in a newly-created table, as shown at block 164, and continues obtaining data for each of the wells. The well_number is incremented by 1, as show at block 166 of FIG. 5. If the well_number is greater than total number of wells (X), then the loop is exited, as shown at block 168 of FIG. 5. Otherwise, the flow chart of FIG. 5 goes to block 154.

The newly created table is then analyzed by the processor 64 in order to determine the suggested parameters for the next experiment.

The initial crystallization parameters such as temperature, time, solvent type, solvent rations, concentration and the yield data obtained by the analyzer for each of the initial experiments are then entered into the program. The program then processes the data, generates multivariable contour maps or response surfaces which describe the behavior of the system of crystallization parameters or variables, and designs a set of new experiments based on the response surfaces. Many variables affect chiral resolution and crystallization of simple organic compounds from reaction mixtures. Typically, for chiral resolution, the temperature of the wells, the types of solvents, the ratio of solvents, the types and concentrations of crystallization partners, and the concentration of the target compound are varied. For crystallization of simple organic compounds from reaction

mixtures, the temperature of the wells, the types of solvents, the ratio(s) of solvents, and the concentration of the target compound are varied.

Methods for studying relationships among multiple parameters and for solving statistical problems related to these relationships are known and include the Monte Carlo method and rotating-simplex method of optimization, otherwise known as the self-directing optimization (SDO) method. A general discussion of the useful statistical methods for solving statistical problems is included in C. Hendrix (1980) Chemtech, August 1980, pp. 488-96 which is incorporated by reference in its entirety. It will be understood by the ordinary skilled artisan that the program may include one or more suitable statistical methods for optimization of processes having multiple parameters and for designing experiments which include multiple variables.

Using a program which utilizes the Monte Carlo method, for instance, the operator can define the space of parameters to be analyzed, run a series of random preliminary experiments in this space, define a new space of parameters using the best of these preliminary experiments, run additional experiments in the new space and continue this process until no further improvement is observed. For example, the operator defines a space of crystallization parameters for each experiment such as crystallization temperature, concentration of starting material, solvent or buffer ratios or concentrations, and time period then performs several preliminary random experiments using the synthesizer. The analyzer data concerning crystallization product yield, for instance, are then stored in the computer as a parameter. Based on the preliminary parameters and the product yield parameter, the program then utilizes the statistical method to generate a new space of parameters (e.g., crystallization temperature, concentration, solvents, reagents and time) for further experimentation. A new set of crystallizations are then performed with the new space of parameters and the result product yield parameter is then stored and processed by the Monte Carlo method as before. This process can be repeated until no further improvements in product yield or purity, for instance, are obtained.

Alternatively, a program which utilizes the SDO method generates a set of experiments in all of the variables of interest for the operator. When these experiment has been run, the experiment that gave the worst result is identified among the set. This

experiment is then discarded and replaced with a new experiment. When the replacement experiment has been run, the worst of the set is again identified and discarded. This process continues until no further improvement is observed. For example, the operator performs preliminary experiments with the synthesizer using SDO variables of interest. For example, the crystallization yield data, in combination with the variables, are then analyzed by the program. The program would then eliminate the experiment with the worst result, e.g., worst yield, and generate a new proposed experiment. This process is repeated until no further improvements in product yield, for instance, are obtained. Alternatively, if the determination of "success" or "failure" is solely based on crystallization purity, the experiment with the lowest purity is discarded and generate a new experiment. In still an alternate embodiment, the "success" or "failure" is based on a variety of factors, such as product yield and purity, so that the experiment with the worst of these factors is discarded.

Another method to analyze the data in the newly created table is by first determining the "weights" for each of the crystallization parameters 172. The crystallization parameters include, for example, the total product yield, the amount of contaminants, the amount of uncrystallized reagents, the time of the crystallization, choice of solvents, and the temperature of the crystallization. Prior to execution of the program, the operator assigns "weights" based on importance of each crystallization parameter. In this manner, the results of each of the wells can be assigned a total "score" by multiplying the crystallization parameters by the "weights" and adding them. For example, if the total product yield and the total time are the two parameters of interest, and the total product yield is considered more important than the time of the crystallization, the "weights" for each can be 0.8 and 0.2, respectively for each of the two parameters. Each of the results for an individual well can then be tallied 174. The well_number is set equal to 1, as shown at block 170. The well_number is incremented by 1, as shown at block 176 of FIG. 5. If the well_number is greater than the total number of wells (X), then the loop is exited, as shown at block 178 of FIG. 5. Otherwise, the flow chart of FIG. 5 goes to block 174. For parameters which are more desirable when they are lower in value, e.g. the time of crystallization, the result of multiplying the weight by the parameter can be inverted, and then added to the total to determine the "score."

The entries can then be arranged based on the score 180. The processor 64 then displays the results of the raw data and the "scores" 182. At each step in the methodology, the display can be updated to inform the operator of the current crystallization. For example, when the processor 64 commands or receives information from the synthesizer 12, the analyzer 40 or the robot 50, the display can be updated to indicate the current operation.

Based on the highest ranked "score," the suggested bounds for the next set of experiments are determined 184, 186. For example, if the temperature of the crystallization is determined to be an important parameter, the temperature value of the highest ranked "score" is used as a base value for the temperature bounds for the next set of experiments. The suggested parameters is then displayed to the operator 188.

This automated process development technology allows a vast array of data to be collected and interpreted. Many combinations of crystallization variables can be investigated in a short time period. Using the current manual technology, only a local optimization is found because it is too time consuming to investigate every set of crystallization conditions. With the new automated technology presented here, a large number of statistical data points can be collected. In essence, a global optimization is found. The amount of data generated by this process is limited only by the number of variables that can be envisioned for a given crystallization.

Some components of the automated technology discussed in this disclosure have found application in combinatorial chemistry for the area of drug discovery. As a result, robotic technology and automated synthesizers, as well as HPLC and LC/MS instruments are commercially available. The novel integration and application of these methods to chemical process research and development, however, has not been pursued to the best of our knowledge.

The hardware elements of the workstation of FIG. 1 are generally known in the art and either commercially available or described in the literature. See, for example, U.S. Pat. Nos. 5,443,791 and 5,463,564 which are incorporated by reference herein. A suitable synthesizer is available from Advanced ChemTech of Louisville, Ky., model no. 4906 MOS and from Bohdan Automation, Inc. of Mundelein, Ill., RAM® synthesizer. Robotic arm 26 mechanisms are incorporated into the automated synthesizer equipment of

Advanced Chemtech and Bohdan Automation. Suitable HPLC and LC/MS analytical instruments equipped with autosamplers are widely available. The synthesizer, robotic arm, and analytical instruments typically come with their own resident computer software, which can be readily modified or augmented by persons of skill in the art to accomplish the chemical process and design of experimentation methodology described herein. A suitable analytical instrument capable of ascertaining purity and structure is the Finnigan MAT (San Jose, Calif.) liquid chromatograph/mass spectrometer (LC/MS/MS).

A general example of the crystallization process relates to potential chiral crystallization partners for kinetic resolution. An extremely wide variety of chiral acids and bases are commercially available, and represent potential chiral crystallization partners for kinetic resolution, one generally "screens" a number of the potential partners in several different solvents, then identifies the trials that provide crystallization. The crystals are examined for stereochemical composition using a variety of methods, including chiral chromatography and optical rotation. This approach can required a long period of time before a suitable crystallization partner and solvent is identified.

Automated liquid handling machinery can be used to aid in the rapid setup of multiple crystallization trials. Generally, a solution of the racemate of interest, in any number of solvents or solvent mixtures, is dispensed into vessels typically consisting of glass wells ranging from 1.8 mL to 20 mL in volume. To those solutions are then added the potential crystallization partners. Using this approach, many combinations of the racemate with x crystallization partners in many solvent mixtures can be tested rapidly. Several hundred experimental trials can easily be set up in a matter of hours.

One point that should be addressed is the choice of the x crystallization partners, including the magnitude of the number x, and the molecular structures of the partners. For example, if the racemate to be resolved contains a carboxylic acid group, one would generally choose a chiral amine to perform the kinetic resolution. However, there are potentially thousands of chiral amines available through commercial sources, and virtually an infinite number available through chemical synthesis.

Computational analysis, particularly diversity analysis, is used to evaluate a large "virtual library" of potential crystallization partners, as shown at block 190 in Figure 6. For example, the structures of 5000 chiral amines are analyzed using a commercial

software application called Diversity Analyzer (which is manufactured by Molecular Simulations, Inc. in San Diego, California), which compares the 5000 chiral amines and provides an output that describes how "similar" or "different" they are with respect to one another. A chemist can then select a smaller library, perhaps containing 100 of the chiral amines, that fairly represents all sections of "diversity space", as shown at block 192 in Figure 6. Thus, in this example, the 100 selected chiral amines would be added to the racemic carboxylic acid, perhaps in three different solvent systems, for a total of 300 test mixtures, as shown at block 194 in Figure 6.

After an appropriate time period, generally 24 hours, the test mixtures are visually inspected for deposition of crystals. Trials that result in crystal formation are selected for analysis. Analysis is rapidly conducted using automated, high-throughput chiral HPLC methodology, which allows detection and quantitation of each enantiomer in the racemic mixture, as shown at block 196 in Figure 6. Generally, the supernatant of trials that resulted in crystal deposition are analyzed using chiral HPLC, to provide an estimate of the enantiomeric resolution that was achieved, if any. Those trials that indicate a high level of resolution are then examined further, which includes collection of the deposited crystals, and evaluation of the enantiomeric purity of the crystalline material by chiral HPLC or optical rotation, as shown at block 198 in Figure 6.

The results of these studies provide the molecular structures of crystallization partners that resulted in crystal formation as well as molecular structures of crystallization partners that did not provide crystals. This information can then be re-evaluated using computational techniques to generate a theoretical model for the types of molecular structures that would afford high levels of chiral separation, as shown at block 200 in Figure 6. Using the "virtual library" that was constructed with the example 5000 chiral amines discussed above, a more focused set of amines can be selected that occupy similar "diversity space" as the amines that were successful in achieving resolution. This focused set of amines would then be used to further optimize the kinetic resolution, using the same process as described for the initial test mixtures. Based upon this analysis, novel crystallization partners can be designed or purchased from commercial sources for optimal chiral separation.

Example 1: Resolution

Stock solutions of 41 different amines (as shown in Table 1) can be prepared in tetrahydrofuran and ethyl acetate (0.12 mmol). A stock solution of ibuprofen in Ethanol (0.12 mmol) is prepared. Two sets of 41 wells (a total of 82 wells) are loaded with ibuprofen solution (2 mL(50 mg)/each vial) using liquid handler and evaporated to dryness.

To the above wells containing ibuprofen (50 mg/vial), the selected amine solutions (5mL/vial) are added from the stock solutions using a liquid handler. The resulting solutions are shaken on an Orbital Shaker at room temperature for 24 hours. The solutions are then visually analyzed for any crystallization. Those wells containing crystals are filtered and the filtrate is analyzed by chiral HPLC to determine enantiomeric enrichment.

Those wells which did not show any crystal formation were cooled in a refrigerator for 24 hours. Again the wells are visually analyzed for any crystal formation, filtered and filtrate was analyzed by chiral HPLC for enantiomeric enrichment.

The reactions that showed selectivity in favor of one or the other enantiomer are selected and studied further for variations in temperature, concentration, addition rate of the amine, solvent, and pH, etc.

The precipitates are also analyzed by chiral HPLC to determine the enantiomeric enrichment in the crystals. The experiments are repeated until a combination that yields highest EE (enantiomeric excess) is found.

Table 1:

count	Amine	MW	MG	MG/5ML
1	(1S,2S,3S,5R)-(+)-ISOPINOCAMPHEYLAMINE	153.2	18.6	92.9
2	(S)-(+)-2-AMINO-5-CYCLOHEXYL-1-PROPANOL HCL	193.7	23.5	117.4
3	(1S,2S)-(+)-2-AMINO-1-PHENYL-1,3-PROPANEDIOL	167.2	20.3	101.4
4	S-(-)-2AMINO-3-PHENYL-1-PROPANOL	151.2	18.3	91.7
5	S-(+)-1-DIMETHYAMINO-2-PROPANOL	103.2	12.5	62.6
6	R-(-)-2-AMINO-1-BUTANOL	89.1	10.8	54.0
7	S-(+)-2-AMINO-1-BUTANOL	89.1	10.8	54.0
8	R-(-)-1-AMINO-2-PROPANOL	75.1	9.1	45.5
9	S-(+)-1-AMINO-2-PROPANOL	75.1	9.1	45.5
10	S-(+)-2-AMINO-1-PROPANOL	75.1	9.1	45.5
11	R-(-)-2-AMINO-1-PROPANOL	75.1	9.1	45.5
12	R-(-)-N-BENZYL-2-PHENYLGLYCINOL	227.3	27.6	137.8
13	R-(-)-2-PHENYLGLYCINOL	137.18	16.6	83.2

14	S-(+)-2-PHENYLGLYCINOL	137.18	16.6	83.2
15	S-(-)-N-BENZYL-ALPHA-METHYLBENZYLAMINE	211.32	25.6	128.1
16	R-(+)-N-BENZYL-ALPHA-METHYLBENZYLAMINE	211.3	25.6	128.1
17	R-(-)-SEC-BUTYLAMINE	73.1	8.9	44.3
18	S-(+)-SEC-BUTYLAMINE	73.1	8.9	44.3
19	S-(-)-N,N-DIMETHYL-1-(NAPHTHYL)ETHYLAMINE	199.3	24.2	120.8
20	R-(+)-N,N-DIMETHYL-1-(NAPHTHYL)ETHYLAMINE	199.3	24.2	120.8
21	R-(+)-N,ALPHA-DIMETHYLBENZYLAMINE	133.1	16.1	80.7
22	S-(-)-N,ALPHA-DIMETHYLBENZYLAMINE	133.1	16.1	80.7
23	R-(+)-ALPHA,4-DIMETHYLBENZYLAMINE	133.1	16.1	80.7
24	S-(-)-ALPHA,4-DIMETHYLBENZYLAMINE	133.1	16.1	80.7
25	S-(-)-N-METHYL-1-(1-NAPHYL)ETHYLAMINE	171.2	20.8	103.8
26	R-(+)-N-METHYL-1-(1-NAPHYL)ETHYLAMINE	171.2	20.8	103.8
27	R-(+)-1-(1-NAPTHYL)ETHYLAMINE	171.2	20.8	103.8
28	S-(-)-1-(1-NAPTHYL)ETHYLAMINE	171.2	20.8	103.8
29	S-(+)-AMINOINDANE	133.19	16.1	80.7
30	S-CYCLOHEX-2-ENYLAMINE	x		
31	R-CYCLOHEX-2-ENYLAMINE	x		
32	S-6-BROMO-2-AMINO TETRALINE HCL	201	24.4	121.9
33	S-4-CHLORO-ALPHA-METHYLBENZYLAMINE	162	19.6	98.2
34	S-4-NITRO-ALPHA-METHYLBENZYLAMINE	177	21.5	107.3
35	R-(+)-N,N-DIMETHYL-1-PHENETHYLAMINE	149.2	18.1	90.5
36	S-(-)-N,N-DIMETHYL-1-PHENETHYLAMINE	149.2	18.1	90.5
37	R-(+)-2-AMINO-3-PHENYL-1-PROPANOL	151.2	18.3	91.7
38	R-(-)-1-CYCLOHEYLETHYLAMINE	127.2	15.4	77.1
39	S-(+)-1-CYCLOHEXYLETHYLAMINE	127.2	15.4	77.1
40	L-(-)-A-METHYBENZYLAMINE	121.1	14.7	73.4
41	D-(+)-A-METHYBENZYLAMINE	121.1	14.7	73.4

Example 2: Crystallization of an Amorphous Solid

Procedure:

The amorphous solid free amine (1.0 g) is dissolved in THF (10 mL) and 100 μl is added to each of the 2mL wells. The solvent is evaporated. Each of the 12 different solvents shown in Table 2 is added to the appropriate wells. The plate is agitated to ensure dissolution of the amine. The 8 different antisolvents shown in Table 2 is added to the appropriate wells at room temperature. The total reaction volumes are 1mL. After the addition of the antisolvent, the wells are visually inspected for solids. Any wells which show no precipitate are cooled to 0°C and are visually inspected for solids. Addition of the antisolvent is at rate of 2mL/minute. All liquid dispensing is done a Gilson 215 liquid handler.

Specifically, 12 solvents and 8 antisolvents at 2 different concentrations are investigated, as shown in Table 2. This allows for the investigation of 192 different crystallization conditions. The test amorphous solid compound (*e.g.*, an amine) may be very soluble in most of the solvents, even with 50% antisolvent. The percent antisolvent/ reaction volume ratio can be changed from 50% and 90% to 85% and 95%.

Table 2: Experimental Array

	Solvent	Antisolvent	%Antisolvent/ reaction vol.
1	ethanol	Pet. ether	85%
2	methanol	MTBE	95%
3	isopropanol	water	
4	THF	isopropylether	
5	chloroform	diethylether	
6	methylene chloride	heptane	
7	dichlorethane	hexane	
8	acetone	pentane	
9	methyethylketone		
10	ACN		
11	toluene		
12	ethylacetate		

The results for the 192 sets of crystallization conditions may be compiled. Specifically, tables displaying the plate map array for each solvent-antisolvent may be compiled. Each cell in the table can represent a vial in the 96 well plates.

In Plate 1, some solvents/antisolvent combinations in the wells in which some precipitate can be observed. The three alcohols, ethanol, methanol, and isopropyl alcohol, for instance, precipitate a milky-white slurry when water is added. The 4th solvent, chloroform, can precipitate some whitish solids with the addition of isopropylether.

In Plate 2, some problems can be observed with ethyl acetate, toluene, acetone, acetonitrile and methylethylketone. Upon addition of the antisolvent, solids could form and quickly become a whitish oil.

The wells in which no precipitate is observed are cooled to 0°C for a few hours. This can produce a few more oily semi-solids. In some cases, the vials are uncovered and some of the solvent is allowed to evaporate. This too can produce oily semi-solids in some of the wells.

For the next set of experiments, those solvents that produced the oily semi-solids in the first set of screening experiments are further analyzed. The amount of antisolvent is varied from 10% to 80% of the total reaction volume. A whitish solid precipitate, which can quickly become an oil, may be observed in some of the wells.

From this last set of experiments, it appears that ethylacetate, toluene and methylethylketone could warrant further investigation. Either hexane or pentane can work equally as well for the antisolvent. Using the range of 40% to 60% antisolvent could bring the solutions to the saturation level. We could then investigate alternative methods to induce crystal growth, such as vapor diffusion or slow solvent concentration at a variety of low temperatures. We could also pursue investigation of the use of alcohols as the solvent and water as the antisolvent. This would be dependent upon examination of the solids we isolated to see whether they are amorphous or crystalline.

It is intended that the foregoing detailed description be regarded as illustrative rather than limiting and that it is understood that the following claims, including all equivalents, are intended to define the scope of the invention.